CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
ANILAZINE

Chemical Code # 000256 Tolerance # 00158
SB 950 # 001
2/26/99
June 11, 1986
Revised July 17, 1986, 1/12/99, 3/17/99

I. DATA GAP STATUS

Chronic toxicity, rat:	Data gap, no study on file
Chronic toxicity, dog:	Data gap, no adverse effect indicated
Oncogenicity, rat:	No data gap, no adverse effect
Oncogenicity, mouse:	No data gap, no adverse effect
Reproduction, rat:	No data gap, no adverse effect
Teratology, rat:	No data gap, no adverse effect
Teratology, rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, no adverse effect
Chromosome effects:	No data gap, possible adverse effect
DNA damage:	No data gap, no adverse effect
Neurotoxicity:	Not required at this time

Toxicology one-liners are attached.

All record numbers through 091209 were examined.
** indicates an acceptable study.
Bold face indicates a possible adverse effect.

File name: t990317
J. Kishiyama & M. Silva, 3/17/99

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may contain additional effects.

COMBINED, RAT

(No study on file)

CHRONIC TOXICITY, RAT

Subacute:
013 034818 "Dyrene: Subacute Oral Toxicity Study on Rats," (Fluke, W. and G. Kaliner; Bayer AG Institute Für Toxikologie, Report #: 7844; 9/29/78). Dyrene (anilazine) Technical (purity = 92%) was administered via oral gavage for 28 consecutive days to SPF Wistar albino rats (20/sex/dose) at concentrations of 0, 30, 100, or 300 mg/kg. Mortality was 5%,10% and 25% for males (300 mg/kg) and females (100 & 300 mg/kg), respectively. The premature deaths were associated with eroded abdominal and thoracic viscera. Body weights were reduced 8-12% for high dose males. Disturbances in behavior (apathy and dyspnea) persisted during the first 3-4 weeks of treatment for males (300 mg/kg) and females ($\geq$ 100 mg/kg)0. NOEL = 30 mg/kg/day. (No work sheets). (Kishiyama & Silva, 3/16/99).

018 011222: Duplicate of 013 034818.

Subchronic:
013 034816 "ANILAZINE: A 13 Week Oral (Dietary Administration) Toxicity Study in the Rat With a 6-Week Interim Kill," (Goodyer, M.J.; Hazleton Laboratories Europe Ltd., Report #: 2660-262/23; 6/81; Mobay #80216). Anilazine (purity not stated) was fed in diet to CD Sprague-Dawley rats for 6 and 13 weeks (5 & 20/sex/dose, respectively) at 0, 500, 2000, or 8000 ppm. NOEL = 500 ppm (There was decreased body weight and food consumption in both sexes at 8000 ppm. Most rats at 8000 ppm had a hunched posture (no longer occurred after week 2). From week 4 onward piloerection, red/brown staining of the fur around the head and back, rhinorrhea and exophthalmos were observed at 8000 ppm. There were increased pathological effects in both sexes at the 6 week and 13 week intervals at $\geq$ 2000 ppm. Histopathology revealed gastric irritation of the stomach, increase in enlarged salivary glands (males at 8000 ppm) and hyperkeratosis/acanthosis of the tail at $\geq$ 2000 ppm. Other effects include decreased creatinine and glucose activity at 8000 ppm.) These data are supplemental. (Kishiyama & Silva, 3/15/99).

086 087116 "ANILAZINE: A 13-Week Oral (Dietary Administration) Toxicity Study in the Rat With a 6-Week Interim Kill," (Goodyear, M.J.; Hazleton Laboratories Europe Ltd., North Yorkshire, England; Mobay No. 80216-1; supplemental date: 6/81). This submission contains an EPA data call in for the subchronic rat study. The data are: gross pathology tables, tissues numbers in histopathology summary tables and a re-representation of the statistical analyses. These data were additional data requested by EPA. A copy, although not specifically requested, was also submitted to DPR. Supplemental to 013 034816. M. Silva, 12/9/98.

013 034814 "Dyrene: Subchronic Inhalation Toxicity Study on Rats," (Mihail, F. and G. Kimmerle; Bayer AG Institute Für Toxikologie, Report #: 7320; 2/17/78; Mobay # 66143). Dyrene (anilazine)

Technical (purity = 92%) in Experiment #1 was administered to Wistar II rats (8-9 weeks old; 10/sex/dose) as an aerosol (6 hours/day;15 days over 3-weeks; 5 times/week) at concentrations averaging 0 (DMSO/polyethelene glycol 400), 0.4, 1.1, 1.8, 2.9, 6.4 or 32.6 mg/m^3 air. Dyrene was tested again in Experiment #2 at concentrations of 0.4, 1.1 and 2.9 mg/m^3 air. In Experiment #1, mortality was 90 - 100% and 10 - 20% at 32.6 and 6.4 mg/m^3 air, respectively. Breathing and mobility disorders were observed at 2.9, 6.4 or 32.6 mg/m^3 air. Body weights were decreased (19-40%) at $\geq$ 6.4 mg/m^3 in both sexes and 6% in females at 1.8 mg/m^3 air. Liver toxicity was indicated by increased SGOT and SGPT activities at 6.4 mg/m^3 air in both sexes. In Experiment #2, body weights were reduced 8% - 15% for both sexes at 2.9 mg/m^3 and 5% for males at 1.1 mg/m^3. Erythrocyte and hemoglobin levels were elevated in males at 2.9 mg/m^3. The NOEL = 0.4 mg/m^3 air (according to the report author). (no worksheet). (Kishiyama & Silva, 3/17/99).

018 011220: Duplicate of 013 034814.

013 034813 "A Study of the Stability and Homogeneity of Anilazine in the Laboratory Animal Diet Preparations," (Grant, C.A.; Hazleton Laboratories Europe Ltd. Report #: 2584-262/27; 1/81; Mobay # 80217). Anilazine preparations of 1000 and 9000 ppm were analyzed for homogeneity and stability analysis. In addition, a supplementary study was performed on 500 and 1000 ppm anilazine for stability analysis. Homogeneity: anilazine content averaged 95.7% and was within ± 20% (80% to 109%) of the target concentration. Stability: anilazine content for 9000 and 1000 ppm samples after 28 days stored at -20°C was 105% and 98% of the respective targets. However; at +20°C, anilazine content was 97% and 80% at 4 days and 92% and 47% at 29 days for the respective 9000 and 1000 ppm target concentrations. A supplemental study performed on 500 and 1000 ppm samples under conditions of housing for rats and showed an anilazine content at 4 days of 82% and 91%, respectively. Based on results it was recommended that diets be prepared weekly, with half the preparation stored at -20°C for the mid-week diet change. (No worksheet). (Kishiyama & Silva, 3/17/99).

CHRONIC TOXICITY, DOG

Subchronic Study:

013 034815 "Dyrene: Subchronic Toxicity Study on Dogs (Thirteen-week oral administration in capsules)," (Hoffmann, K. and P. Gröning; Bayer AG, Institut Für Toxikologie, Report #: 8199; 2/22/79; Mobay #: 68035). Dyrene (92% pure) was administered to Beagle dogs (4/sex/dose) in a single daily oral dose in gelatin capsules at concentrations of 0 (empty gelatin capsules), 50, 150, or 450 mg/kg body weight for 13 weeks. NOEL = 50 mg/kg (Pulse rate was reduced for both sexes at $\geq$150 mg/kg at week 13. The increase in the Incidence of diarrhea and vomiting was dose related in both sexes but was most pronounced at $\geq$150 mg/kg. Body weight values are reduced for males (11%) and females (19%) at termination (Week 13) at 450 mg/kg. There was decreased albumin/globulin quotient at $\geq$ 150 mg/kg in both sexes during weeks 7 and 13. Total protein was reduced for high dose females. Ovary weight was significantly reduced at 450 mg/kg. Thymus weight was significantly reduced at 450 mg/kg. Thymic atrophy was increased in males at $\geq$ 150 mg/kg. All treated males and most females showed Increased diffuse round cell infiltration of the esophagus. A slight incidence of inflammatory cell infiltration of the stomach was observed at 450 mg/kg.) No adverse effect. These data are supplemental. (Kishiyama & Silva, 3/16/99).

085, 087 085021, 091208, 091221 "Subchronic Toxicity Study on Dogs," (Hoffmann, K. And Groning, P.; Bayer Ag, Institut fur Toxikologie, Mobay #: 68035-3, supplement date 2-22-79). 158-

085 085021 This submission contains restructured hematology and clinical chemistry tables for samples taken at 0, 7 and 13 weeks for control and groups 1, 2 and 3. This is a copy of additional data requested by EPA. 158-087 091208 (Mobay #: 68035-2): this submission contains the analytical results of the test compound utilized in the subchronic dog study. Purity of the analytical material was 92.8 - 93.2%. 158-087 091221 This addendum provides a detailed GLP statement by the study director. In this statement the author refers to Bayer Report #: 8199 (same as Mobay Corporation #: 68035). There is also an explanation of how the analyzed samples relate to the test material, as requested by reviewers at EPA. **Supplemental** to 013 034815. M. Silva, 12/9/98.

018 011221. Exact duplicate of 013 034815.

Chronic Studies:

014, 080, 087 010143, 070197, 091195, 091206-7, 091210 "Dyrene Chronic Toxicity to Dogs on Oral Administration (18 Month Capsule Study)," (Hoffmann, K., Luckhaus, G. and Janda, B.; Bayer Ag, Bayer Ag, Fachbereich Toxikologie, Mobay No. 84123-3, 3-5-83, addendum to Bayer Report #: 11613, 3/7/83). Anilazine (91.5 - 94.4% pure) was administered orally (by capsule) to Beagle dogs (4/sex/dose) at 0, 10, 40 and 160 mg/kg for 18 months. NOEL systemic = 10 mg/kg, body weight gain decrease, loose stools, vomiting. **No adverse effects.** Bayer Ag retrospectively conducted histological exams on tissue from dogs treated at 10 and 40 mg/kg Dyrene (control and high dose histopathology had already been performed [080 070197]). Previously reviewed as unacceptable and possibly upgradeable (Schreider, 4/9/85), upon submission of the requested information (summary of gross lesions and histopathology incidence summary, as well as individual histopathology for each dog, individual clinical observations, food consumption, ophthalmology, test article data & QA/GLP), with re-evaluation of the study it remains unacceptable. Reviewers now consider to study to be **not upgradeable** (dogs were treated with numerous drugs before and during treatment with dyrene). M. Silva, 12/9/98.

087 091218. Duplicate of 091206.

087 091195 Mobay #: 84123-4; Analytical results; comment on appearance of test material in feces.

087 091206 Mobay #: 84123-5; Response to EPA comment.

087 091207 Mobay #: 84123-2; Correlation of gross pathology and histopathology.

087 091210 Mobay #: 84123-1; Stability data, GLP statement and selected individual data.

ONCOGENICITY, RAT

010 035447 "Bioassay of Anilazine for Possible Carcinogenicity-Rats," (National Cancer Institute, NIH 78-1354, 1978) Anilazine, no purity stated, lot No. 4050279 was fed in the diet to Fischer 344 rats for 103 weeks at 0, 500 or 1000 ppm, 25/sex in controls, 50/sex/group exposed to test article. Systemic NOEL < 500 ppm, no increase in tumor frequency reported. **No adverse effect. Unacceptable. Not upgradeable.** Insufficient number of controls, only two exposure levels with inadequate high dose, individual data not provided, no hematological data presented, no analysis of dosing material, no analysis of test article, all appendices of data are missing. J. Schreider,

4-1-85. Reexamination of the study for body weight effects supports the statement that the high dose was inadequate and finds that the weight effect noted in males only is not sufficient evidence for an m.t.d. Therefore, the NOEL noted as less than 500 ppm in the first review is changed. No other clinical or microscopic effects were reported. NOEL > 1000 ppm. J. Gee, 7-17-86.

** 014 010142 "Anilazine 104 Week Oral (Dietary Administration) Carcinogenicity and Toxicity Study in the Rat" (Hazleton Laboratories Europe Ltd., Mobay No. 86861, 4-84) Anilazine, 93.5%, was fed in the diet to Sprague Dawley rats for 2 years at 0, 50, 330 or 2000 ppm, 50/sex/group and 10/sex/group for hematology. NOEL = 330 ppm, body weight. There were almost no effects at high dose (decreased weight gain in the first few weeks) but other data indicate that higher dose would cause stomach erosion. **No oncogenic effect. Acceptable** as on oncogenicity study but inadequate for a combined. J. Schreider, 4-1-85 and J. Gee 6-13-86.

Addendum to 010142: 084, 087 072398, 091213, 091214, 091215, 091219, 091222, (084 072838--exact duplicate of 087 091214): "Anilazine 104-Week Oral (Dietary Administration) Carcinogenicity and Toxicity Study in the Rat," (Goodyer, M.J.; Hazleton Laboratories Europe Ltd., North Yorkshire, UK; Laboratory Project ID #: 86861, 4/84). 084 072398 Mobay Report #: 86861-1 This volume contains dose selection justification. Rat eyes were examined for histopathological lesions, in lieu of ophthalmology (no remarkable findings). 087 091213 This volume provides an analysis of anilazine technical along with purity data (90.8 - 93.5% pure). 087 091214 Provides a list of the DPR-requested information and a statement that, since there were no effects in the urinalysis or ophthalmology in the subchronic study, the chronic study should be acceptable. 087 091222: This volume contains a duplicate of the purity data and a detailed GLP statement by the study director (study #: 262/22; same as Mobay Corp. #: 86860. 087 091215: This volume contains the justification for high-dose selection in the long-term rat and mouse bioassays of anilazine. Included was the NCI Bioassay of Anilazine for Possible Carcinogenicity (1978), previously reviewed by DPR (volume/record #: 158-010/035447). The chronic rat study remains unacceptable and not upgradeable (no MTD, no effects at any dose, no ophthalmology or urinalysis). The possibility of an adverse chronic effect cannot be determined, since an MTD was not reached in this study. These data are supplemental. M. Silva, 1/5/99.

084 072838 Supplementary to 014 10142. "Justification for High-Dose Selection in Long-Term Rat and Mouse Bioassays of Anilazine," (Rieth, J.P., Mobay Corporation, Stilwell, KS; Laboratory Project ID Report #: 98283; 9/14/88). This volume stated that "The doses used in the long-term studies of Anilazine on rats (Goodyer, 1984) and mice (Goodyer, 1984) sponsored by Bayer AG (performed at Hazleton Laboratories Europe, Ltd.) Were selected based on the NCI report as well as on range-finding studies (also performed at Hazleton) specifically designed for that purpose." In the rat range-finding study (Goodyer, 1981), rats were fed Anilazine, in diet at 0, 500, 2000 or 8000 ppm (25/sex/dose) for 6 and 13 weeks (see DPR volume/record #: 158-013 034816). The sacrifice at 6 weeks was 5/sex/dose and the rest were sacrificed at 13 weeks. At 13 weeks, males showed 9% decreased bodyweight gain (2000 ppm), accompanied by gastric lesions in 27% of the rats. Because of these effects and because 2000 ppm was twice the dose used in the NCI study (see DPR volume/record #: 158-010/035447). Summary only, DPR prepared no worksheet. M. Silva, 1/5/99.

CONCLUSION: The combined study remains acceptable for oncogenicity only. No data have been presented which can be evaluated for the possible upgrading of the chronic portion of a study. The doses used did not illicit chronic toxicity.

ONCOGENICITY, MOUSE

010 919864 "Bioassay of Anilazine For Possible Carcinogenicity - Mice" (National Cancer Institute by Gulf South Research, NIH 78-1354, Mobay #: 98283, 1978) Anilazine, purity not stated, lot No. 4050279, fed in the diet to B6C3F1 mice for 103 weeks at 0, 500 or 1000 ppm and observed for 4-6 weeks, 25/sex in the control, 50/sex/group exposed to test article. NOEL < 500 ppm. **No oncogenic effects. Unacceptable, not upgradeable.** (insufficient number of control animals, only summary data presented, only two exposure levels, individual data not provided, no hematological data presented, no analysis of diet except at 8 weeks, no analysis of test article, all appendices are missing) J. Schreider, 4-1-85. Reexamination of the study for body weight effects as a justification of dose and establishment of a NOEL finds that the lower body weight in males only after about week 20 is not adequate for a high dose. In addition, the decrease is not dose dependent but approximately the same for both 500 and 1000 ppm. The only data, however, is a figure presenting the means and it is difficult to determine the actual percentage of the decreased gain. The same report contains information on a 13-week subchronic study in which no body weight effect was noted up to 8000 ppm. The NOEL stated above is changed to NOEL > 1000 ppm (HDT). J. Gee, 7-17-86.

087 091215 Mobay #: 98283: Dose justification for 010 919864.

087 091228 Mobay #: 86860-1: Analysis of test article; Mobay #: 86860-2: GLP statement for 011195.

015 011195 "Anilazine: 104 Week Oral (Dietary Administration) Carcinogenicity and Toxicity Study in the Mouse" (Goodyear, M.J.; Hazleton Laboratories Europe Ltd., Mobay No. 86860, 4-84) Anilazine, 93.5%, was fed to CD-1 mice at 0, 50, 250 or 1250 ppm for 2 years, 50/sex/group, satellite group for hematology. No signs of toxicity at high dose but 6-week feeding study in mice supported the selection of the high dose for an onco study (severe toxicity at 3000 and 9000 ppm). Inadequate for a chronic study. **No oncogenic effect. Acceptable with variances (no clinical observations or time to tumor). J. Schreider, 4-8-85.

013 034817 Dose Range-Finding Study (supplement to 011195): “Anilazine: A 6 Week Oral (Dietary Administration) Dose Range-Finding Study in the Mouse,” (Taupin, P.J. Y.; Hazleton Laboratories Europe Ltd. Report No.: 2600-262/21; Mobay #: 80215; 6/81). Anilazine (purity not stated) was administered in diet to Swiss derived CD-1 mice (5/sex/dose) at concentrations of 0, 1000, 3000 or 9000 ppm for 6 weeks. The 9000 ppm dose was cancelled by the end of the second week due to mortality and/or deaths *in extremis*. Body weights were decreased 21% - 30% (NOTE:10% lower week 0) and 3% to 15% decreased for both sexes at 3000 ppm. Red blood cell count was decreased for females at 3000 ppm. Necropsy revealed dark gastrointestinal contents (2, 2 and 6 at low, mid and high doses, respectively). Gastric erosin was observed in some of the animals from all treated groups. Due to the lack of definitive treatment related effects at 1000 ppm, a dietary concentration of 1250 ppm was selected for the high dose in the definitive, long term chronic/oncogenicity study. (No worksheet). (Kishiyama & Silva, 3/17/99).

084 072838 Supplementary to 015 011195 “Justification for High-Dose Selection in Long-Term Rat and Mouse Bioassays of Anilazine,” (Rieth, J.P., Mobay Corporation, Stilwell, KS; Laboratory Project ID Report #: 98283; 9/14/88). This volume stated that “The doses used in the long-term

studies of Anilazine on rats (Goodyer, 1984) and mice (Goodyer, 1984) sponsored by Bayer AG (performed at Hazleton Laboratories Europe, Ltd.) Were selected based on the NCI report as well as on range-finding studies (also performed at Hazleton) specifically designed for that purpose." In a rangefinding study, mice (5/sex/dose) were treated at 0, 1000, 3000 and 9000 ppm for 6 weeks. All mice dosed at 9000 ppm died. Males at 3000 ppm had a statistically significant decrease in bodyweight gain. The report speculated that the decrease in bodyweight gain may have been due to to chemical irritation of the gastrointestinal tract (severe enough to induce bleeding). In the NCI study, 1000 ppm was an MTD (decreased bodyweight gain). At 3000 ppm, potentially life-threatening gastrointestinal ulceration occurred. Therefore, a high dose of 1250 ppm was selected for the chronic mouse study. This information is supplemental. No data were presented (summary only)--No worksheet. M. Silva, 1/5/99.

REPRODUCTION, RAT

**034 026966 "Anilazine: 3 Generation Oral (Dietary Administration) Reproduction Study in the Rat" (Hazleton Laboratories Europe Ltd., Mobay No. 88887, 11-84) Anilazine, 93.5%, fed in the diet to Sprague-Dawley rats for a 3 generation, 1 litter/generation study at 0, 50, 330 or 2000 ppm, based on 13-week study, 15 males/group, 30 females/group. NOEL = 330 ppm, body weight gain in F_0 males. Diet analysis at intervals, decreased indices of mating (92.9% in control and 79.3% in high dose) and fertility (86.7% in control and 76.7% in high dose) at high dose, confined to first generation (F_0). Initial evaluation by Bankowska, 6-19-85, noted this as a possible adverse effect. Reevaluation by Gee and Parker, 6-16-86, considers this effect not to be of biological significance because it did not appear in the F_1 or F_2 matings. In addition, the fecundity index (defined as pregnant/mated x 100) was not effected in the F_0 mating. Mating index: number of copulations/number of estrus cycles required x 100. Fertility index: number pregnant/total number of females. Acceptable. No significant biological effect on reproduction.

086, 087 087117, 091212 "ANILAZINE: 3-Generation Oral (Dietary Administration) Reproduction Study in the Rat," (Irvine, L.F.H.; Hazleton Laboratories Europe Ltd., UK; supplemental date, 11/84; Report #: 88887-2). Addendum 086/087117: Physical development data for neonates in each generation were presented in graphic form. They were also submitted in numerical form (summarized, as well as individual data) so that a comparative evaluation (intra- and inter-generation) of the measured parameters could be completed. Individual data for clinical signs in parental animals were included (Appendix 2). Included was a statement by the study director that this study was conducted in conformance with FIFRA GLP Guidelines, even though it was conducted prior to GLP requirements. The report author refers to Study #: 3744-262/26, which is the same study reported by Mobay Corporation as #: 88887. Addendum 087/091212 contains 3 parts: An analysis of the test article used in the reproduction study (purity = 90.8 - 93.5%). A report on the effects of Dyrene on the contractile ability of the isolated rat uterus (briefly mentioned on page A1.28 of the Dyrene reproduction study.) and a reevaluation of the effect of anilazine on mating and fertility during the F0 generation. The conclusion was that anilazine did not affect mating and fertility. This conclusion was also reached by DPR reviewers. These data are supplemental (no worksheet). M. Silva, 12/9/98.

TERATOLOGY, RAT

015 011203 "Dyrene Evaluation for Embryotoxic and Teratogenic Effects on Orally Dosed Rats"

(Machemer, L.; Bayer Ag, Toxicology, Biochemistry and Pharmacokinetics, Mobay No. 66534, 8-21-78) Anilazine, 94%, was given to Long Evans rats by gavage on days 6-15 of gestation at 0, 30, 100 or 300 mg/kg, 20 females/group. NOEL (maternal) = 100 mg/kg, weight gain. NOEL (developmental) = 300 mg/kg. **Unacceptable. Possibly upgradeable** (inadequate information on test article, no convincing evidence that MTD was achieved, no analysis of dosing solution, no individual data, only 1/3 for visceral exam). **No adverse effect** in the report but insufficient information for an independent determination by review without the individual data. Schreider, 4-3-85.

** 083 072247 "A Teratology Study with Anilazine (Dyrene Technical) in the Rat," (Kowalski, R.L., Clemens, G.R. and Hartnagel Jr., R.E.; Toxicology Department, Miles, Inc., Mobay No. 98360, 9-30-88). Anilazine (Dyrene technical, batch 87R0244F, 97.4% pure) was administered by gavage to mated and presumed pregnant Crl:CD®BR rats for days 6-15 of gestation at 0 (aqueous Emulphor), 150, 500 or 1500 mg/kg (28/dose). **Maternal NOEL =** 150 mg/kg (Maternal toxicity was characterized by a dose dependent increased incidence of soft stool (28.6%--500 mg/kg & 100% at 1500 mg/kg). Body weights and body weight gain were significantly reduced on days 10, 12, 15 and 20 (body weight) and throughout the study (weight gain) at 1500 mg/kg. Food consumption was statistically decreased at 500 and 1500 mg/kg on day 7. Gross examination revealed an increase in incidence of thickened and coarse mucosa in the nonglandular part of the stomach at 500 (5/22--17.9%) and 1500 mg/kg (4/23--14.8%). Histopathologically, epithelial hyperplasia (acanthosis) and hyperkeratosis of the mucous membrane was observed. There was also, in some animals either mucosal erosion or ulcer formation and submucosal inflammation characterized by infiltration of lymphocytes and eosinophils. **Developmental NOEL =** 1500 mg/kg (HDT) **No adverse effects. Acceptable.** M. Silva, 12/9/98.

TERATOLOGY, RABBIT

** 084 072399 "Teratology Study with Anilazine (Dyrene Technical) in the Rabbit," (Clemens, G.R. and Hartnagel Jr., R.E.; Toxicology Department, Miles Inc., Mobay No. 98466, 9-30-88). Anilazine technical (batch 87R0244F, 97.4% pure) was administered by gavage to artificially inseminated American Dutch rabbits (20/dose) at 0, 15, 40 or 75 mg/kg on days 6 - 18 of gestation. **Maternal NOEL** < 15 mg/kg (There were increased deaths and abortions at $\geq$ 15 mg/kg. Anilazine produced a statistically significant decreased maternal body weight gain at $\geq$ 40 mg/kg (days 6-18). Body weight gain was also significantly decreased days 0-28 and actual body weight gain at 75 mg/kg. Absolute body weights were significantly decreased days 18 and 21 at 75 mg/kg. Food consumption was sporadically significantly reduced for all groups during treatment. The decrease was dose dependent and at 75 mg/kg food consumption was most greatly decreased.There was a dose dependent increase in clinical signs, namely small or no stools, nasal discharge, reddish discharge, dyspnea and whole body twitching. Nasal discharge was observed in 5% of control animals and in 10, 45 and 40% of low, mid, and high dose animals, respectively. **Developmental NOEL =** 40 mg/kg (At 75 mg/kg fetotoxicity was displayed by a statistically significant increase in fetal mortality, lower fetal weights and delayed skeletal ossification. The increase in fetal mortality along with increased resorption in 1 litter (not linked with maternal health) resulted in an increase in post-implantation loss at 75 mg/kg. Body weights of fetuses were decreased at 75 mg/kg. Five fetuses from three litters at 15 mg/kg had fused or abnormal skull bones.) **No adverse effects. Acceptable.** M. Silva, 12/14/98.

015 011202 "Dyrene Evaluation for Embryotoxic and/or Teratogenic Effects on the Rabbit After Oral Administration" (Roetz, R.; Bayer AG, Toxicology, Biochemistry and Pharmacokinetics, Mobay No. 80322, 4-11-81) Anilazine, 93.4%, was given by gavage on days 6-18 of gestation to Himalayan rabbits at 0, 5, 15 or 45 mg/kg, 11-13 pregnant females/group. NOEL not determinable from limited data. **No teratogenic effect** stated in the report. **Unacceptable** (inadequate information on test article, no analysis of dosing solution, no corpora lutea counts, no individual data, beginning body weights not presented, no convincing evidence that MTD achieved), possibly upgradeable. Insufficient data for independent evaluation by review. Schreider, 4-3-85.

GENE MUTATION

Microbial Systems

** 063 064929 "Anilazine Tech. (The Active Ingredient of Dyrene) *Salmonella*/Microsome Test for Determination of Point Mutations," (Herbold, B.; Bayer AG Fachbereich Toxikologie, Mobay No. 94852, 5-18-87) *Salmonella typhimurium* strains TA1535, TA100, TA1537 and TA98 were assayed with Anilazine technical (batch 233 969 362, 97.1% pure) at concentrations from 2.5 to 400 µg/plate. The assay was plated in quadruplicate (repeat trial, plated to toxic levels). There was no increase in mutagenicity (revertant colonies). **No adverse effects. Acceptable.** M. Silva, 12/4/98.

087 091211 This submission is supplemental to 063 064929: "Anilazine Tech. (The Active Ingredient of Dyrene) *Salmonella*/Microsome Test for Determination of Point Mutations," (Herbold, B.; Bayer AG Fachbereich Toxikologie, Mobay No. 94852-1, 5-18-87). The submission contains analysis of technical test material (purity range: 97.1 - 97.4%). No worksheet. M. Silva, 1/5/99.

015 011200 "Dyrene *Salmonella*/Microsome Test for Detection of Point-Mutagenic Effects" (Herbold, B.; Bayer AG, Toxicology, Biochemistry and Pharmacokinetics, Mobay No. 69365, 5-20-80) Anilazine, 95.9%, tested with and without metabolic activation on *Salmonella typhimurium* strains TA1535, 1537, 100 and 98. Trial 1: 0, 20, 100 or 500 µg/plate, Trial 2: 0, 5, 15, 45, 135 or 405 µg/plate. No increase in reversion frequency reported. **Unacceptable** (inadequate controls in runs without S9, high variability in each run, duplicate plate data not presented), not upgradeable. Schreider, 4-3-85.

015 011198 "Mutagenicity Evaluation of Dyrene in the *Saccharomyces* Reverse Mutation Assay: Revised Final Report" (Jagannath, D.R., Study Director. Litton Bionetics Inc., Mobay No. 80348, 8-17-81) Anilazine, purity not stated, tested with and without activation on *S. cerevisiae* strains S138 (frameshift mutant) and S211 (base pair mutant), measuring reversion to methionine prototrphy. Trial 1 (3-hour inclubation): 1, 10, 100, 500, 1000 or 2500 µg/tube with and without S9. Each tube contained 0.05 ml test solution, 0.1-0.2 ml yeast and 0.5 ml buffer or S9. Trial 4 (1-hour incubation): 10.5, 26.3, 52.5, 75, 105 or 150 µg/tube. **Increased mutation frequency** in both strains 100 to 500 µg/tube with and without activation in the presence of extreme cytotoxicity (either not measured or <15% viability so that the results for genotoxicity are confounded by the cytotoxicity). When the trial 4 was conducted at lower concentrations and increased survival, no increase in reversion was detected. This trial however, used only a 1-hour incubation time so is not entirely comparable with the previous trials. **Unacceptable, not upgradeable** (excessive contamination, positive control not effective with and without S9 in several trials, single value only for each concentration). Reevaluation by Gee finds that the study is too inadequate to determine

the significance of any adverse effect. Schreider, 4-4-85, Gee, 6-16-85.

087 091194 Addendum to record #: 011198. "Mutagenicicty Evaluation of Dyrene (Bayer Study No. T 002846) in the *Saccharomyces* Reverse Mutation Assay," (Jagannath, D.R.; Litton Bionetics Inc., Mobay #: 80348-1; 10/81). Data on the purity of the test compound as used in the original study was approximately 93.5%. No worksheet. (Kishiyama & Silva, 1/5/99).

087 091217 Addendum to record #: 011198. "Mutagenicicty Evaluation of Dyrene (Bayer Study No. T 002846) in the *Saccharomyces* Reverse Mutation Assay," (Jagannath, D.R.; Litton Bionetics Inc., Mobay #: 80348-2; 10/81). Additional information on the test article purity and characterization was provided. No worksheet. (Kishiyama & Silva, 1/5/99).

Mammalian Systems

** 094 089666: "Detection of Gene Mutation in Somatic Mammalian Cells in Culture: HGPRT-Test with V79 Cells," (Heidemann, A., Bayer AG Department of Toxicology, Friedrich-Ebert-STR., Report No. R 3821, Laboratory Project ID Report No. 100575. January 31, 1986). Anilazine technical (approximately 97.4% pure) was used on Chinese Hamster V79 cells in an HGPRT gene mutation test (Experiments I & II) at 0.2, 1.0,1.5, 2.0 µg/ml (no S9 Mix) or 0.9, 7.0, 8.0, or 9.0 µg/ml (+ S9 Mix) with a 4 hour exposure. A third experiment was performed with doses of 1.0, 1.5, 2.0, 2.25, or 2.5 µg/ml (no S9 Mix) and a 4 hour exposure. The increased mutation rate observed in experiment I was not confirmed in later experiments. ACCEPTABLE, No adverse effect. (Kishiyama & Silva, 12/1/98).

063 064927 "Dyrene Spot Test on Cross-Bred C57B1/6J x T Stock Mouse Embryos to Evaluate for Induced Somatic Changes in the Genes of the Coat Pigment Cells" (Herbold, B.; Bayer Ag, Institute of Toxicology, Mobay No. 94408, 9-2-86) Dyrene (batch 233496419, 97.3% pure) was given to pregnant mice (C57B1/6J females were mated with T males) by i.p. route on day 10 of gestation at 0, 3, 10 or 30 mg/kg. At least 300 F1 animals were produced in order to obtain meaningful results in a mammalian spot test. The purpose was to test for induction of somatic gene changes in coat pigment cells in F1 offspring *in utero* after treatment of dams. The incidence of relevant coat spots (RS) was not increased for treated animals. **No adverse effects. Unacceptable, not upgradeable** (high dose was too low). M. Silva, 1/5/99.

** **015 011196:** "Mutagenicity Evaluation of Anilazin Technical in the Mouse Lymphoma Forward Mutation Assay Final Report (Revised)" (Witterland, W.F., Study Director; Litton Bionetics, Mobay No. 86725, 3-11-84) Anilazine, 95.9%, tested with and without activation on L5178Y mouse lymphoma cells; 2.5-9.0 mg/ml without S9, 1.0-5.0 mg/ml with S9; 2 day expression time; 3 dishes/concentration for cloning at 200 cells/dish; **positive** for dose related mutagenicity at concentrations showing cytotoxicity. **Acceptable.** Gee, 4-8-85.

CHROMOSOME EFFECTS

080 070196 "Dominant Lethal Test on the Male Mouse to Evaluate for Mutagenic Effects," (Herbold, B.A., Bayer Ag, Fachbereich Toxicology, Mobay No. 97409, 5-6-88). Dyrene (batch 233 696 362, 97.3% pure) was given to groups of 50 Bor:NMRI male mice at 0 (0.5% aqueous Cremophor emulsion), 5000 or 7500 mg/kg. Males showed acute symptoms for 72 hours including apathy, reduced motility, roughened fur, spasm and diarrhea. Mortality showed 3/54 died at 5000

mg/kg and 3/54 died at 7500 mg/kg. Males were mated with females 1:1 for 4 day periods (12 periods total). No confirmation of mating was performed. Females were sacrificed 14 days after presumed mating to evaluate number of live and dead implantations and corpora lutea. In each time period 29 -41 females were pregnant/group. No dominant lethal effect was observed. **No adverse effects. UNACCEPTABLE.** Upgradeable (appropriate positive control data). M. Silva, 12/8/98.

**** 063 064926** "Dyrene Cytogenetic Study with Human Lymphocyte Cultures in Vitro To Evaluate for Harmful Effect on Chromosomes," (Herbold, B.; Bayer Ag, Institute of Toxicology, Mobay No. 91774, 1-7-86). Human lymphocytes from 1 male and 1 female were placed in culture and were exposed to technical grade Dyrene (batch 233 496 419, 97.7/97.3%), with and without metabolic activation, at concentrations of 0 (DMSO), 3.0, 10.0 or 30.0 µg/ml. There were 4 cultures per concentration; mitotic index determined from 1000 cells/culture, 200 metaphase cells scored per concentration. Dyrene caused a significant reduction in mitotic index to 60% of controls at 30 µg/ml without S9 and a significant reduction to 20% of controls at 30 µg/ml with S9. With and without S9 mix there was a dose response increase in aberrations (breaks, gaps, exchanges, fragments and multiple aberrations). This increase was also observed when gaps were excluded. There was a significant increase in polyploid cells in 400 metaphases (no S9) at $\geq$ 10 ug/ml (not observed with S9). With activation, the increase in aberrations was significant at all concentrations (not statistically significant without S9). **Possible adverse effect. Acceptable.** M. Silva, 12/2/98.

** 079 070151 "Anilazine Micronucleus Test on the Mouse for Clastogenic Effects," (Herbold, B.; Bayer AG Toxicology Department, Mobay No. 97475, 1-3-88). Anilazine technical (batch 233 696 362, 97.1% pure) was given by gavage in a single dose to NMRI mice at 7500 mg/kg. Samples of bone marrow were taken at 24, 48 and 72 hours. The negative (vehicle) and positive (cyclophosphamide, 20 mg/kg) controls were sampled only at 24 hours. All groups had 5/sex/time point. No indication of a clastogenic effect. **No adverse effects. Acceptable.** M. Silva, 12/8/98.

087 091216 “Anilazine: Micronucleus Test on the Mouse for Clastogenic Effects,” (Herbold, B.A., Bayer AG Toxicology Department, Mobay Report #: 97475-1, 1/3/88). This volume is a supplemental submission and contains information on the analysis and characterization of the test article used in this study. No worksheet. (Kishiyama & Silva, 1/5/99).

** 063 064928 "Chromosome Aberration Test in Bone Marrow Cells of the Chinese Hamster with Anilazine Technical," (Herbold, B.; Cytotest Cell Research, Mobay No. 94787, 5-14-87). Anilazine technical (batch 233 696 362, 97.1% pure) was given by gavage to Chinese hamsters (6/sex/dose, with 2/sex additional at 3000 mg/kg in case of deaths) at 0 (0.5% Methocel), 300, 1000 or 3000 mg/kg in a single dose. Bone marrow samples were taken for all dose levels after 24 hours and at 6 and 48 hours for 3000 mg/kg (5/sex/time point analyzed; 50 metaphase cells/animal scored). No increase in chromosome aberrations under test conditions. No adverse effect, acceptable. Silva, 12/3/98.

015 011201 "Dyrene Dominant Lethal Study on Male Mouse to Test for Mutagenic Effects" (Herbold, B.; Bayer AG, Toxicology, Biochemistry and Pharmacokinetics, Mobay No. 66127, 4-10-78) Anilazine, no purity stated, tested in a dominant-lethal assay on NMRI mice at 0 or 500 mg/kg (single oral dose), 50 males/group, 600 females/group. Mate 1:1 over 12 pairings. **No adverse effect reported. Unaccptable, not upgradeable** (inadequate information on the test article, no positive controls, single dose may not have been high enough, no individual obs,

appendices not included, tables could not be interpreted due to lack of translation from German). Schreider, 4-3-85.

018 011219 Duplicate of 015 011201.

015 011199 "Dyrene Anilazine Micronucleus Test on Mouse to Evaluate Dyrene for Mutagenic Potential" (Herbold, B.; Bayer AG, Toxicology, Biochemistry and Pharmacokinetics, Mobay No. 69366, 11-24-80) Anilazine, 95.5%, tested in the micronucleus assay on NMRI/W77 mice at 0, 100 or 200 mg/kg with positive control group, 2 doses at 24 hour intervals, 5/sex/group. **No evidence of clastogenic effects. Unacceptable** (two dose levels only, only one sacrifice time at 6 hours after last dose, no statistical analysis). In a preliminary test at 2 x 250 mg/kg, 1/5 mice died so high dose may be adequate. 1000 PCE's were evaluated and NCE/PCE determined. Schreider, 4-3-85.

DNA DAMAGE

032 026965 "Dyrene-Anilazine POL A1-Test on E. coli to Evaluate for DNA Damage" (Bayer AG, Toxicology, Biochemistry and Pharmacokinetics, Mobay No. 80213, 8-26-81) Anilazine, 93.5%, tested with and without activation on E. coli strains P3478 and W3110 at 62.5, 125, 250, 500 or 1000 µg/plate with positive controls, 4 plates/concentration. **No adverse effect** reported on zones of inhibition. **Unacceptable** (no justifications of concentrations used, no individual values, incomplete information on how assay performed). Gee, 9-9-85.

015 011197 "Rat Hepatocyte Unscheduled DNA Synthesis Assay" (B.C. Myhr, Study Director; Litton Bionetics Inc., Mobay #86428, 8-16-83) Anilazine, 98.6%, tested in UDS assay on Fischer 344 rat primary hepatocytes at 0.049, 0.098, 0.20, 0.49, 0.98, 2.0 or 9.8 mg/ml with positive control. Excessive toxicity above 9.8, 150 nuclei evaluated/concentration. **No adverse effects, acceptable. Gee, 4-8-85.

NEUROTOXICITY

No studies on file.

ADDITIONAL STUDIES

087 091209, 091220 “Dyrene, Acute Toxicity Studies,” (Flucke, W.; Bayer AG, FRG, Laboratory Project ID #: 53102-1, 53102-2; 4/77). This addendum to BAYER Report #: 6761 (4/77) contained summary tables. **Table 1**: Acute Oral Toxicity to Rats (LD50 > 5000 mg/kg). At ≥ 500 mg/kg both sexes had behavioral disturbances. At ≥ 1000 mg/kg, both sexes showed diffiuclt breathing and diarrhea. **Table 2:** Acute Oral Toxicity to Rats (Fasted). Males showed mortality (1/15) after 1 day at 5000 mg/kg and females showed 2/15 died at 4000 mg/kg (3 d), 5/15 at 4500 mg/kg (1-4 d) and 13/15 at 5000 mg/kg (1-3 d). Signs were: behavioral disturbances (both sexes, all doses); Difficult breathing (M & F at ≥ 500 mg/kg) and Diarrhea (M & F ≥ 1000 mg/kg). **Table 3:** Acute Oral Toxicity (LD50) to Mice: At 2500, 3500 & 4000 mg/kg mortality increased (M = 1/15, 3/15 & 7/15; F = 1/15, 8/15 & 12/15 at 1-5 d). Behavioral disturbances (both sexes, all doses) and difficult breathing (M ≥ 1000 mg/kg & F ≥ 2500 mg/kg) occurred. **Table 4:** Acute Intraperitoneal Toxicity to Rats: LD50 = 71 mg/kg. Behavioral disturbances (all doses), difficult breathing (≥ 25 mg/kg),

hunched back & stiff to halting gait (≥ 50 mg/kg), diarrhea & swollen abdomen (60 - 100 mg/kg) occurred in both sexes. **Table 5:** Acute Subcutaneous Toxicity to Mice: LD50 = 3512 mg/kg (F). Behavioral disturbances (all doses) & difficult breathing (≥ 1000 mg/kg-M & ≥ 2000 mg/kg-F) were observed. **Table 6:** Acute Percutaneous Toxicity (LD50) to Rats: LD50 > 5000 mg/kg. Behavioral disturbances and reddening of skin for 1-3 d occurred in both sexes at 5000 mg/kg (only dose tested). No bodyweight effects were observed in any test with either species. A GLP was contained in 091220. These data are supplemental. M. Silva, 1/11/99.

018 011229 "Dyrene Acute Toxicity Studies," Bayer Report 6761; Mobay #53102; 4/77. Not reviewed.

083 072248 "Excretion and Metabolism of [^{14}C]-Dyrene® in Rats," (Lee, S.G.K., Wood, S.E., Delk, J.L. and Pither, K.M.; Mobay Corporation, Stilwell, Kansas; Laboratory Project ID #: DR4R; Mobay Project ID Report #: 98396; 11/14/88). Dyrene as triazine-UL-^{14}C was administered orally (gavage) in a single dose ([^{14}C]-Dyrene at 5 mg/kg; presumptive NOEL or 500 mg/kg, high dose) or multiple gavage doses (5 mg/kg for 14 consecutive days, followed by a single dose of [^{14}C]-Dyrene) to both sexes of Sprague Dawley rats (6/dose). A 4th group was gavaged with [^{14}C]-Dyrene of a higher specific activity at 5 mg/kg to facilitate identification/characterization of metabolites in urine and feces. Dyrene was eliminated primarily in the feces (90%) and urine (10%) by 72 hours. No IV, group A included due to low water solubility of anilazine. The primary metabolite in feces was dihydroxy Dyrene and in urine were: dihydroxy Dyrene and dimercapturic acid conjugate of Dyrene. These data are supplemental. M. Silva, 1/5/98.